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TRANSLATION FROM RUSSIAN. SHIRYAEV, D. T., SHEVCHENKO, S. F., TOKAREV, S. A., and OREKHOVA, I. M.* (1966). Experimental study of Hyalomma plumbeum plumbeum Panz. and Haemaphysalis punctata Can. and Fanz. ticks as tularemia vectors. Med. Parasit., Moscow, 35(3):305-309.

A rather rich material has accumulated in literature on natural infection of different tick species with the tularemia agent. However, experimental testing of their importance as vectors of this infection, which allows us to estimate the role played by ticks in maintaining tularemia epizootics, has been studied only in certain species. Thus, Petrov (1959) and Olsufjev (1960) cite 14 ixodid tick species in which natural tularemia infection was demonstrated. The ability to maintain and transmit this microbe under experimental conditions has been demonstrated in only 8 tick species (Golov, 1934; Golov, Fedorov, 1934; Olsufjev, Tolstukhina, 1941; Olsufjev, 1943; Shatas and Bystrova, 1954; Petrov, Dunayeva, 1955; and Shevchenko, 1958, and others).

In the present article, the authors' main aim was to study susceptibility, maintenance, and transmission of the tularemia agent by Hyalomma p. plumbeum and Haemaphysalis punctata ticks. These ticks are widely distributed in the southern steppe zone of USSR, and were found repeatedly in natural conditions to be infected with tularemia (Shatas and Bystrova, 1954; Pilipenko, Derevyanchenko, 1955; and Shevchenko, 1960, and others).

MATERIAL AND METHOD.

Ticks reared in the laboratory from noninfected females were utilized in the experiment. Tick cultivation was made by Olsufjev (1941), and Pospelova-Shtrom's (1941) methods. Engorged and hungry specimens were kept in special test tubes (see Instruction for Parasitological Work, 1959) at 16°-33°C. Engorged females oviposited and embryonic development occurred in the same conditions. Larval tests were made 74-96 days after hatching. Prior to tests, hungry larvae were kept over 2 months in a refrigerator at 4°C, and this prolonged their life. Larvae were removed

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from the refrigerator 1 to 2 days prior to tests. In one test, larvae of the second species were placed on animals for feeding 13 to 26 days after hatching.

In all tests carried out on different animals, H. p. plumbeum behaved as a two-host type. Before larvae were placed on animals, detached engorged nymphs were removed. Engorged nymphs detached from the animal 13 to 23 days after beginning bloodsucking as larvae. Immature stages required 15 days (average) to complete engorgement. Adult stages of this tick species were placed on hosts 11 to 40 days after molting. H. punctata nymphs were placed on animals 12 to 21 days after molting. Tests with adult ticks of this species were incomplete because molting of engorged nymphs just commenced as this work was put into official form. Engorgement of H. punctata larvae continued for 6 to 9 days, and of nymphs 7 to 11 days.

For the purpose of infecting larvae, white mice and guinea pigs were used as donors. A freshly isolated strain Fr. tularensis No. 1567, was used to infect them. The animals were infected 5 to 6 days prior to completion of bloodmeal, thus the most favourable conditions for their infection was obtained, because by this time the animals usually died from generalized infection.

In cases in which the animal's death occurred before H. p. plumbeum nymphs were completely engorged, the latter were transferred to healthy animals for completing engorgement. This proved that incompletely engorged nymphs and adult ticks are capable of attaching to the animal and to complete engorgement 5 to 82 days after interrupted bloodsucking.

A total of 5,730 larvae, 1,517 nymphs, and 328 adults of both species, 364 white mice, 13 guinea pigs, and 1 rabbit were utilized in this investigation.

1. Hyalomma plumbeum plumbeum.

For testing the possible infection of the larval stage and subsequent transmission of the agent in the progress of metamorphosis, 1 test was made on a guinea pig which became infected 6 days after larval attachment. Four days later the guinea pig died of tularemia, just at the moment when engorged larvae were ready to molt on the animal. However after the death of the guinea pig, in spite of a great number of engorged larvae only 88 larvae molted into nymphs within 3 days. Biological investigation of 17 engorged larvae gave positive results (Table 1).

In subsequent tests, infection of ticks was done in the nymphal stage on guinea pigs and white mice. Nymphs usually commenced to ingest

the tularemia agent 2 to 3 days prior to the death of animals and particularly during the agonal period. However, in spite of generalized infection in animals, we did not record a 100% nymphal infection (Table 1).

For testing transstadial transmission of the agent, nymphs and adult ticks of this species were investigated. Infection of two white mice was done with suspensions prepared from 47 hungry nymphs obtained from molted infected larvae that fed for 3 to 5 days on an infected animal; both mice died of tularemia on day 4. Group investigation of adult ticks infected at various periods showed that the tularemia agent may be transmitted from nymphs to adults. Investigation of individual adult ticks showed that 64.3% were infected (Table 2).

Biological examination of dead, incompletely engorged nymphs kept 1 to 9 days at room temperature 19°-25°C, and that were infected 83-115 days prior to their death, gave negative results. In examination of dead hungry adult ticks infected in the nymphal stage 109-131 days prior to their death, the same results were obtained. When dead ticks were kept 9 to 60 days in a refrigerator at 4°C, further investigations demonstrated that the tularemia agent survived.

Together with elucidation of transmission of the agent during the progress of metamorphosis, we also studied possible infection of recipients during a bloodmeal. Thus, when 41 hungry nymphs were placed on two healthy white mice 9 to 14 days after molting and 13-17 days after infection, both mice died of tularemia on day 4 or 5. Incompletely engorged nymphs removed from infected animals and transferred to 10 healthy white mice are able to attach to the animals 5 to 82 days after feeding was interrupted. Transmission of the tularemia agent to mice occurred in 50% of cases, while infection in other mice did not occur, since nymphs placed on these animals proved to be sterile in biological investigation.

Tests on transmission of this infection during a bloodmeal by adult ticks were done on 4 guinea pigs and 4 white mice. All guinea pigs died from day 11 to 16, but it was possible to isolate the tularemia agent only from two of the guinea pigs. Ticks removed from one of the dead guinea pigs, in which the tularemia agent was absent, also proved to be infected.

As regards white mice, all died in 4 to 17 days, but a tularemia culture was isolated from only 1 mouse. Biological investigation of ticks removed from mice in which tularemia infection was not found showed infection in two cases. Negative results obtained at examination of animals on which ticks proved to be infected may be explained by the

considerable decomposition of their carcasses, and that further passaging was not made. Thus, 6 of 8 animals were infected with tularemia when adult ticks were fed on them. Data on infection of animals when infected ticks were fed on them are presented in Table 3.

In one case, we observed infection of a white mouse with tularemia after eating 10 infected nymphs.

Ability of ticks to transmit the agent to their progeny was verified in a small amount of material. In the tests, progeny from 3 infected females removed from a horse in May 1962 on Biryuchem Island of Khersonsk region were utilized. For testing transovarial transmission, 38 bioassays with 3,420 larvae, 403 nymphs, and 80 adult ticks were made. Negative results were obtained in all tests. In addition, the possibility of transovarial transmission was tested by feeding the progeny from infected females on a rabbit. The results also were negative.

2. Haemaphysalis punctata.

Infection of the larval stage was accomplished by feeding on infected guinea pig and a white mouse. Placing of larvae and infection of the guinea pig occurred simultaneously, considering the terms of the animal's death and duration of bloodsucking in this stage. The guinea pig died after 6 days, and a total of 2,150 larvae in various engorgement degree were collected. Most larvae were not viable and quickly died. Thus, a total of 1,453 larvae died when they were kept for 65 days in a refrigerator at 4°C. However, hungry larvae kept in identical condition remained alive for over a year.

Control tests on larval infection was made 70 days after feeding on a guinea pig. Two bioassays were made with 491 live larvae in various engorgement degrees, and 4 bioassays with 1,453 dead larvae. White mice inoculated with suspension of live larvae died of tularemia. In 1 test with dead larvae, a positive result was also obtained (Table 1).

The second larval group (of about 2,000) was infected on a white mouse. A total of 62 larvae attached and the others died. Feeding commenced 3 days prior to infection of the mouse. Investigation of 26 engorged larvae removed from the animal the day after infection and 1 day prior to death of animal showed that they were infected (Table 1).

Of the remaining living 242 engorged larvae, 80.2% molted into nymphs. Thus, we were able to obtain a small number of potentially infected nymphs. Verification on transstadial transmission of infection was made in only 1 test. One white mice inoculated with a suspension prepared from 7 hungry nymphs died from tularemia on day 7.

The remaining nymphs were kept alive by feeding on laboratory animals, thus allowing us to work out the problem of maintenance of the agent in each stage and ability of ticks to transmit infection to animals during bloodsucking. For this purpose, 2 to 53 nymphs were placed on 5 white mice and 1 guinea pig 101 to 119 days after sucking blood of an infected animal. Four mice died of tularemia (Table 3), in one case engorgement of 3 nymphs was sufficient for infection of a mouse. Biological investigation of nymphs removed from a mouse and a guinea pig, which previously gave negative result, showed their sterility.

Biological investigation of a female infected in the larval stage 262 days prior to the test, gave positive results for tularemia.

We did not study transovarial transmission in these ticks, but there are data in literature (Golov, 1935) that such transmission has not been obtained.

Conclusion

1). H. p. plumbeum and H. punctata infected in the larval stage are able to retain the tularemia agent during the progress of metamorphosis.

2). H. p. plumbeum and H. punctata nymphs and adults transmit tularemia microbes to susceptible animals during bloodsucking.

3). After interrupted feeding, H. p. plumbeum nymphs are able to reattach and infect laboratory animals for as long as 82 days (observation period).

4). Transovarial transmission in H. p. plumbeum was not obtained.

5). Experimental data allows us to assume that H. p. plumbeum and H. punctata ticks in the southern part of the steppe zone in USSR, where they are numerous, may be of significant importance in maintaining natural tularemia foci.

Summary (Original in English)

According to literature, ticks Hyalomma plumbeum plumbeum and Haemaphysalis punctata naturally infected with tularemia occur in nature. The authors established that under experimental conditions tick of both species infected in the larval stage could retain the agent throughout the metamorphosis. Nymphs and imago of H. plumbeum and nymphs of H. punctata during blood-sucking infect animals susceptible to tularemia. Besides, nymphs of H. plumbeum can attach and transmit the causative agent to animals in interrupted feeding for 82 days. No transovarial transmission could be obtained in H. plumbeum ticks.

Experimental evidence suggests that *H. plumbeum* and *H. punctata* ticks in the southern part of the steppe zone of the USSR, where they are prevalent, can have some importance in maintaining foci of tularemia.

TABLE 1.

Results of tick infection when fed on animals infected with tularemia.

Tick species	Animal species	Infection stage	Number of ticks examined	Number of bioassays	Number of cultures isolated
H. p. plumbeum	Guinea pig	Larvae	17	1	1
	White mice	Nymphs	68	3	3
	Guinea pig	"	193	21	8
H. punctata	Guinea pig	Larvae	1944	6	3
	White mouse	"	26	2	2

TABLE 2.

Transstadial transmission of tularemic agent in ticks.

Tick species	Tick stages	Period from the moment of infection (in days)	Number of ticks examined	Number of bioassays	Number of cultures isolated
H. p. plumbeum	Nymphs	4-5	47	2	2
	ADULTS				
	Single (females	33-142	15	15	8
	Single (males	33-53	13	13	10
	In groups (females	33-123	22	3	1
	In groups (males	33-123	31	3	1
	Without division into sexes	66-75	31	4	4
H. punctata	Nymphs	161	7	1	1
	Adult females	262	1	1	1

TABLE 3.

Results of animal infection by feeding infected ticks on them.

Tick species	Tick stages	Tick number		Period from the moment of infection (in days)	Number of animals			
		placed	attached		Guinea pigs	White mice	Infected	Noninfected
H. p. plumbeum	Hungry nymphs	41	39	13-17		2	2	
	Incompletely engorged nymphs	265	196	2-82		10	5	5
	Adults	(58	51	43-60	4		3	1
		(27	26	57-73		4	3	1
H. punctata	Hungry nymphs	177	65	101-106	1	5	4	1
		10	8	119	1			1